compliance with the broad dictates of 35 U.S.C. § 112. Favorable consideration of claims 1, 6-17 and 22-47 are respectfully requested.

The specification has been amended to correct a factual error in that the identification of constituent saccharides for gum tragacanth listed on Table 3 of the application is incorrect. Specifically, as is well know in the art, gum tragacanth contains galacturonic acid, galactose, fucose, xylose, arabinose, and rhamnose rather than galacturonic acid and sialic acid, see the excerpt from the *Merck Index* attached as Exhibit A and the excerpt from the *Handbook of Water-Soluble Gums and Resins* attached as Exhibit B. It is respectfully submitted that the constituent saccharides of gum tragacanth are well known by those of ordinary skill in the art and Applicants therefore request that the amendment of the specification be entered into the application.

Claims 1, 8-10 and 15-17 stand rejected under 35 U.S.C. §112, second paragraph, for indefiniteness. Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Claims 8, 10, 15, and 17 have been amended to overcome the rejection under 35 U.S.C. §112. The pending Office action did not state a rejection of claim 1 under 35 U.S.C. §112. For the foregoing reasons it is respectfully requested that the rejection of claims 1, 8-10 and 15-17 under 35 U.S.C. §112, second paragraph, be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

Claim 1 stands rejected under 35 U.S.C. §102(b) over Japanese Patent Reference No. 57007420 to Yamasa Shoyu Co., Ltd. ("Yamasa '420"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Attached as Exhibit C is a complete English translation of Yamasa '420 prepared by Technical Translation Service of Willoughby, Ohio. As noted therein, Yamasa '420 discloses an antitumor agent that contains as an active ingredient a cell wall component of mold belonging to the *Aspergillus* genus. As is well known in the art, cell walls contain saccharides and in the case of the mold *Aspergillus oryzae*, Yamasa '420 discloses that the cell walls contain saccharides including glucose, mannose, galactose, ribose, arabinose, glucosamine and galactosamine.

Yamasa '420 discloses that the cell walls of *Aspergillus oryzae* were analyzed by hydrolyzing the cell walls with sulfuric acid and then neutral sugars were quantified by the Petri

method and amino sugars were quantified by an amino acid automatic analyzer. The results of such analysis indicated that the cell walls included a neutral sugar quantity of 61% made up of glucose, mannose, galactose, ribose and arabinose, an amino sugar quantity of 19.9% made up of glucosamine and galactosamine and a crude protein quantity of 7.4% for a total of 88.3% - leaving the remaining 11.7% undefined and unidentified. It is important to note that the saccharide content of the cell walls of *Aspergillus oryzae* were identified as a result of the analysis of such cell walls.

In contrast, the antitumor agent that contains as an active ingredient a cell wall component of mold belonging to the *Aspergillus* genus does not result in the hydrolyzation of such cell walls to yield individual bioavailable saccharides. Instead, according to Yamasa '420, the cell wall component is prepared for administration in accordance with known cultivation, separation and refinement methods. The cultivation methods disclosed by Yamasa '420 include liquid cultivation methods such as liquid surface cultivation methods, in-liquid cultivation methods including shaking, cultivation and deep cultivation as well as solid cultivation methods using agar, nylon paste, asbestos or sponge as the cultivation base, as well as mixed cultivation methods in which the two are combined. The mold is then separated and removed from the cultivation matter by "ordinary methods" such as centrifugal separation, filtration, tilting or pressure rods. The mold is then pulverized by physical methods using a homogenizer, dynomill or a French press. The cell walls are then prepared from the pulverized mold by elution of the contents in the mold. Yamasa '420 discloses that water, salt solutions, acids, organic solvents and surfactants can be used alone or in combinations thereof as the eluate. According to Yamasa '420 the cell wall component is obtained by centrifugal separation or filtration of the mold suspension, and the eluate is removed by rinsing or dialysis. Finally, the cell wall component, in a form to be used as the active ingredient, is obtained by drying the rinsed cells by spray drying, air drying, freeze drying or vacuum drying. The cell wall component can also be further refined by sterilization such as by boiling, evaporation, drying, irradiation and ultraviolet rays.

Accordingly, Yamasa '420 discloses a cell wall component of mold that upon hydrolysis is revealed to include various saccharides. Contrary to the claimed composition, which provides nutritionally effective amounts of nutritional product saccharides, the cell wall component of Yamasa '420 includes covalently bound saccharides, which are not bioavailable.

Moreover, claim 1 has been amended to specify that the claimed dietary supplement compositions comprise at least six saccharides selected from a group of sixteen saccharides. Yamasa '420 does not disclose or suggest a dietary supplement composition that comprises at least six such saccharides.

Accordingly, for the foregoing reasons, it is respectfully submitted that Yamasa '420 does not disclose or suggest the claimed dietary supplement compositions. The rejection of claim 1 under 35 U.S.C. §102(b) over Yamasa '420 should therefore be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

Claims 1 and 6-7 stand rejected under 35 U.S.C. §102(b) over U.S. Patent No. 3,890,438 to Cayen et al. ("Cayen '438"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Cayen '438 discloses pharmaceutical compositions for lowering blood cholesterol that include a mixture of diosgenin or a related diosgenin derivative and a 4-substituted phenoxyisobutryic acid or an ester or salt thereof. Cayen '438 discloses that suitable pharmaceutical formulations include tablets comprising: (a) the above-noted compositions, (b) known pharmaceutical carriers and excipients such as starch, sugars and lubricants, suspensions or syrups comprising the above-noted compositions, and (c) suspending agents such as water soluble gums.

Contrary to the claimed dietary supplement compositions, however, Cayen '438 does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, for the foregoing reasons, it is respectfully submitted that Cayen '438 does not disclose or suggest the claimed dietary supplement compositions. The rejection of claims 1 and 6-7 under 35 U.S.C. §102(b) over Cayen '438 should therefore be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

Claims 1, 6, 9-10 and 18-19 stand rejected under 35 U.S.C. §102(e) over U.S. Patent No. 5,202,122 to Graves et al. ("Graves '122"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Graves '122 discloses a process for enhancing the natural bile acid binding capacity of edible pulp material, which is also referred to as dietary fiber. Graves '122 discloses that the major constituents of dietary fiber include cellulose, hemicellulose, lignin and pectin. Graves '122 also discloses at column 6, lines 37-42 that pectin comprises the neutral sugars D-galactose, L-arabinose, D-xylose and L-fucose.

Contrary to the claimed dietary supplement compositions, however, Graves '122 does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, for the foregoing reasons, it is respectfully submitted that Graves '122 does not disclose or suggest the claimed dietary supplement compositions. The rejection of claims 1, 6, 9-10 and 18-19 under 35 U.S.C. §102(e) over Graves '122 should therefore be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

Claims 1, 6-7 and 20-21 stand rejected under 35 U.S.C. §102(b) over Japanese Patent Reference No. 59112922 to Endoh ("Endoh '922"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Attached as Exhibit D is a complete English translation of Endoh '922 prepared by Technical Translation Service of Willoughby, Ohio. As noted therein, Endoh '922 discloses a blood sugar reduction agent that contains as an active ingredient a saccharide selected from a group of polysaccharides that are difficult to digest produced by plants or animals, and derivatives thereof. Endoh '922 discloses several examples of polysaccharides that are difficult to digest. Endoh '922 further discloses implementation examples of the blood sugar reduction agents that are comprised of a single one of the difficult to digest polysaccharides.

Contrary to the claimed dietary supplement compositions, however, Endoh '922 does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, for the foregoing reasons, it is respectfully submitted that Endoh '922 does not disclose or suggest the claimed dietary supplement compositions. The rejection of claims 1, 6-7 and 20-21 under 35 U.S.C. §102(b) over Endoh '922 should therefore be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

Claim 11 stands rejected under 35 U.S.C. §103(a) over Endoh '922 or Cayen '438 in view of U.S. Patent No. 4,260,603 to Pegel et al. ("Pegel '603"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Claim 11 depends from and includes all of the subject matter of Claim 1. The deficiencies of Endoh '922 and Cayen '438 with respect to the subject matter of Claim 1 are noted above and are equally applicable to claim 11.

Pegel '603 discloses a medicament having prostaglandin-synthetase inhibiting activity. The medicament is disclosed to contain as an active principle sterolglycosides and/or their esters and/or spiroketal steroid glycosides and/or esters thereof. Contrary to the Office action, Pegel discloses at Column 5, lines 1-38 a process for the production of sitosterol – ß – D – glucoside not a sitosterol - ß –glucoside of diosgenin. Also, in contrast to the claimed dietary supplement compositions, Pegel '603 does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, even if it would be proper to combine the disclosures of Endoh '922, Cayen '438 and Pegel '603, the combination would not result in the claimed dietary supplement since none of the references disclose or suggest a dietary supplement composition that comprises at least six saccharides.

For the foregoing reasons, it is respectfully submitted that Endoh '922 or Cayen '438 in view of Pegel '603, alone or in combination, do not disclose or suggest the claimed dietary supplement compositions. The rejection of claim 11 under 35 U.S.C. §103(a) over Endoh '922 or Cayen '438 in view of Pegel '603 should therefore be withdrawn.

Claims 12-17 stand rejected under 35 U.S.C. §103(a) over Graves '122, Endoh '922 and Cayen '438 in view of U.S. Patent No. 5,607,693 to Bonte et al. ("Bonte '693") and further in view of The "Prescription for Nutritional Healing" by Balch et al. ("Balch"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Claims 12-17 depend, directly or indirectly, from and include all of the subject matter of Claim 1. The deficiencies of Graves '122, Endoh '922 and Cayen '438 with respect to the subject matter of Claim 1 are noted above and are equally applicable to claims 12-17.

Bonte '693 discloses a cosmetic composition for stimulating hair growth, retarding hair loss or combating pruritis, which includes as an active ingredient, a cosmetically effective amount of

oxyacanthine. In certain embodiments, the composition may also include a saponin. In contrast to the claimed dietary supplement compositions, however, Bonte '693 does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

The Balch reference consists of several extracts from a work entitled "Prescription for Nutritional Healing: A Practical A to Z Reference to Drug-Free Remedies Using Vitamins, Minerals, Herbs & Food Supplements." The Balch reference appears to be a general guide to the bodily function and source of a multitude of vitamins, herbs and food supplements. Balch indeed discloses that vitamins are essential to life and that every living cell on the planet depends on minerals for proper function and structure, however, Balch does not attribute such a lofty status to the antioxidant melatonin. Furthermore, in contrast to the claimed dietary supplement compositions, Balch does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, even if it would be proper to combine the disclosures of Graves '122, Endoh '922, Cayen '438, Bonte '693 and Balch the combination would not result in the claimed dietary supplement since none of the references disclose or suggest a dietary supplement composition that comprises at least six saccharides.

For the foregoing reasons, it is respectfully submitted that Graves '122, Endoh '922, Cayen '438, Bonte '693 and Balch, alone or in combination, do not disclose or suggest the claimed dietary supplement compositions. The rejection of claims 12-17 under 35 U.S.C. §103(a) over Graves '122, Endoh '922 and Cayen '438 in view of Bonte '693 and further in view of Balch should therefore be withdrawn.

Claims 8-9 stand rejected under 35 U.S.C. §103(a) over Graves '122 in view of U.S. Patent No. 5,308,838 to McAnalley et al. ("McAnalley '838"). Insofar as it may be applied against the present claims, this rejection is respectfully traversed.

Claims 8-9 depend, directly or indirectly, from and include all of the subject matter of Claim 1. The deficiencies of Graves '122 with respect to the subject matter of Claim 1 are noted above and are equally applicable to claims 8-9.

McAnalley '838 discloses that acemannan, the active component of the purified ethyl alcohol extract of the inner gel of the leaves of *Aloe barbadensis* Miller, has direct stimulatory effects on the immune system and directly interacts with virus or other infectious organisms,

infected cells, and tumor cells. McAnalley '838, however, does not disclose or suggest a dietary supplement composition that comprises at least six saccharides.

Accordingly, even if it would be proper to combine the disclosures of Graves '122 and McAnalley '838, the combination would not result in the claimed dietary supplement since neither of the references disclose or suggest a dietary supplement composition that comprises at least six saccharides.

For the foregoing reasons, it is respectfully submitted that Graves '122 and McAnalley '838, alone or in combination, do not disclose or suggest the claimed dietary supplement compositions. The rejection of claims 8-9 under 35 U.S.C. §103(a) over Graves '122 in view of McAnalley '838 should therefore be withdrawn. When read in light of the specification, Applicant submits that none of the foregoing amendments narrow the claims with regard to the claimed dietary supplement.

For all of the foregoing reason, it is respectfully submitted that claims 1, 6-17 and 22-47 are in condition for allowance. Favorable reconsideration and allowance of claims 1 and 6-17 and favorable consideration and allowance of claims 22-47 are respectfully requested.

Respectfully submitted,

Date: 7/9/01

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#465743 v2; 013258.0172

ATTACHMENT A
MARKED-UP VERSION

In the Specification

The following paragraph, beginning on page 11, line 6, and ending on page 11, line 24, is amended as indicated below. Additions to the specification are shown with double underlining, so as to distinguish such additions from the text that appeared underlined in the specification as filed.

Table 3. Natural sources of saccharides.

Source Carbohydrate	Corresponding Saccharide(s)
gum tragacanth	galacturonic acid, galactose, fucose, xylose, arabinose and rhamnose [galacturonic acid and sialic acid]
guar gum	mannose and galactose (1:2 molar ratio)
rice or grain flour	glucose
LAREX B-1000 (Larch tree extract)	polyarabinogalactan
MANAPOL™ (aloe vera extract)	acetylated mannose based polymer
gum ghatti	arabinose, galactose, mannose, xylose, glucuronic acid (10:6:2:1:2 molar ratio)
starch	glucose
pectin	galacturonic acid
chondroitin sulfate	N-acetylgalactosamine
chitin	N-acetylglucosamine

Source Carbohydrate	Corresponding Saccharide(s)
acacia, gum arabic	arabinose, galactose, glucuronic acid
alginic acid	mannosyluronic acid, gulosyluronic acid
carrageenan	galactose, 3,6-anhydrogalactose
dextran	glucose
xanthan gum	glucose, mannose, glucuronic acid

The following paragraph, beginning on page 12, line 5, and ending on page 12, line13, is amended as indicated below.

As used herein, the term "carbohydrate" is used interchangeably with the terms "saccharide", "polysaccharide", "oligosaccharide" and "sugar" the definitions of which are well known in the art of carbohydrate chemistry. Although the compositions of the invention are intended to include at least one of the eleven essential saccharides, it should be noted that the saccharides can be in the form of mono-, oligo- and/or polysaccharides, e.g. a composition containing gum tragacanth and guar gum will be considered as containing galacturonic acid, [sialic acid], fucose, xylose, arabinose, rhamnose, mannose and galactose. Therefore, by controlling the amount of particular gums in a given dietary supplement, one can control the amount of the respective saccharides in said dietary supplement.

The following paragraph, beginning on page 17, line 3, and ending on page 17, line 14, is amended as indicated below.

EXAMPLE 1

A suitable composition for a product according to the present invention is as follows: tragacanth gum (100 kg), a source of galacturonic acid [and sialic acid (N-acetylneuraminic acid)] ,galactose, fucose, xylose, arabinose and rhamnose is charged into a stainless steel ribbon

blender and guar gum (10 kg), a source of mannose and galactose, is charged into the stainless steel ribbon blender. The mixture of tragacanth gum and guar gum is mixed for five (5) minutes. Then 250 grams of Aerosil 380™ (silica gel) is added to the mixture as a flowing agent and 200 kilograms of rice flour, a source of glucose, is added as a gluten-free filler. The mixture is then agitated for fifteen (15) minutes. Finally, 100 grams of calcium stearate is added to the mixture as a lubricant and the mixture is agitated for an additional three (3) minutes to generate a bulk powder. The powder is then encapsulated into size 1 gelatin capsules at a fill weight of 250 mg using a Model 8 (Elanco) capsule filling machine.

In the Claims

The following claims 1, 8, 10, 15, and 17 are amended as indicated below.

1. (Twice Amended) A dietary supplement for providing nutritional product saccharides in monomeric, oligomeric or polymeric and derivatized or underivatized form, which saccharides are essential components of glycoproteins in a mammal, said dietary supplement comprising [a composition consisting of] nutritionally effective amounts of:

at least [one saccharide] six saccharides selected from [a first] the group [of saccharides] consisting of:

galactose, glucose, mannose, xylose, [and] acetylated mannose, [; and at least one saccharide selected from a second group of saccharides consisting of:] N-acetylneuraminic acid, fucose, N-acetylgalactosamine, N-acetylglucosamine, arabinose, glucuronic acid, galacturonic acid, iduronic acid, [and] arabinogalactan, glucosamine and galactosamine.

8. (Twice Amended) A dietary supplement according to claim 1 further comprising a nutritionally effective amount of a blend [consisting] of [ripened and] freeze-dried and powdered raw fruits and vegetables.

10. (Amended) A dietary supplement according to claim 8, wherein said blend [consisting] of [ripened and] freeze-dried and powdered raw fruits and vegetables comprises:

broccoli, brussel sprouts, cabbage, carrot, cauliflower, garlic, kale, onion, papaya, pineapple, tomato and turnip.

15. (Twice Amended) A dietary supplement according to claim 1 further comprising nutritionally effective amounts of a dioscorea complex and a blend [consisting] of [ripened and] freeze-dried and powdered raw fruits and vegetables.

17. (Amended) A dietary supplement according to claim 16, wherein:

said vitamins comprise A, B1, B12, B2, B6, beta carotene, bioflavanoids, biotin, C, choline, D, E, folic acid, inositol, K, niacinamide, para-aminobenzoic acid, and [panthothenic] pantothenic acid; and

said minerals comprise boron, calcium, copper, GTF chromium, iodine, iron, magnesium, manganese, molybdenum, potassium, selenium, silicon, vanadium [,] and zinc.

THE MERCK INDEX

AN ENCYCLOPEDIA OF
CHEMICALS, DRUGS, AND BIOLOGICALS

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Merck Research Laboratories
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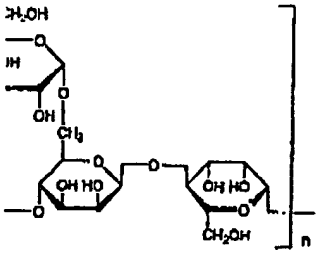
1996

l monohydrate, $C_{19}H_{12}N_2O_9PNa_2.H_2O$, h
ds. decomp at about 250°. Characteris
(molar absorbancy): 13.7 × 10^3 at 25
y in water at 25° about 25 g/100 ml. Ph
lcohol, acetone, ether.
odium salt as flavor intensifier, like sodi
odium glutamate. Said to be more effect

an. Principal polysaccharide from end
seeds, *Cyamopsis tetragonaloba* (L.) Tau
leyne, Whistler, *J. Am. Chem. Soc.* 70, 27
re: Whistler, Durso, *ibid.* 74, 5140 (19
Koleske, Kurath, *J. Polymer Sci.* Pt. A,
rview: Deuel *et al., Chimia* 8, 64 (1954).



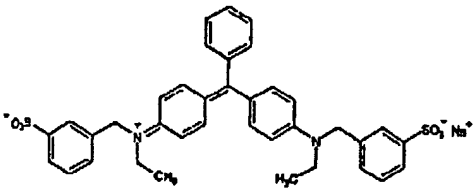
NaOH). Sol in cold water.
ous material, mp 226-227°. Can be forme
which can be elongated 550%. Becom
does not develop crystallinity.
and paper industry.

Gum. Guar flour; gum cyamopsis; cy
ntonite V-7-E; Jaguar; Decorpa; Guarim
em. Mol wt about 220,000. The ground
yamopsis tetragonolobus (L.) Taub., Leg
, cultivated in India as livestock feed. Th
ction (85%) of guar flour is called guara
of linear chains of (1→4)-β-D-mannopyra
α-D-galactopyranosyl units attached b
Ratio of D-galactose to D-mannose is 1:
metabolism: D. J. A. Jenkins *et al., Br*
(1979); on glucose and lipid levels in di
y volunteers: U. Smith, G. Holm, *Athero*
n, Ire.) 45, 1 (1982); on renal tumors i
C. Chin *et al., Biomed. Res.* 5, 273 (1984
er in patients with non-insulin dependen
McIvor *et al., Am. J. Clin. Nutr.*, 41, 89
gy studies: S. L. Graham *et al., Food Ca*
, 287 (1981). Comprehensive monograph
ontgomery, *The Chemistry of Plant Gum*
einhold, New York, 1959) 627 pp. *Revie*
in *Industrial Gums*, R. L. Whistler, Ed
, New York, 2nd ed., 1973) p 303-321.
powder. Completely sol in cold and ho
y insol in oils, greases, hydrocarbons, k
ater solns are tasteless, odorless, nontoxic
fluorescent gray color, and neutral. Stable
eight times the thickening power of starch
be converted to a gel by small amounts
ersions are neutral. *Cf.* "A Comparativ
ercially Available Guar Gums" by J. A
J. Bartilucci, *Drug Standards* 25, 149-15
mnic, female rats (g/kg): 7.35, 6.77 oral

sizing; as a protective colloid, stabilize
um forming agent for cheese, salad dre
oups; as a binding and disintegrating agen
tions; in pharmaceutical jelly formulation
emulsions, lotions, creams, toothpastes;
stry as a flocculant, as a filtering agent;
as a coagulant aid.
Adjunct to diet, insulin or oral hypogl
l of diabetes.

a Green B. *N-Ethyl-N-[4-[[4-[ethyl[(3-s
[amino]phenyl]phenylmethylene]-2,5-cyc*

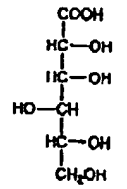
hexadien-1-ylidene]-3-sulfobenzenemethanaminium inner salt, sodium salt; C.I. Acid Green 3; C.I. Food Green 1; FD & C Green 1; C.I. 42085. $C_{37}H_{35}N_2NaO_6S_2$; mol wt 690.82. C 64.33%, H 5.11%, N 4.06%, Na 3.33%, O 13.90%, S 9.28%. Prepn: Jones *et al., J. Assoc. Offic. Agr. Chem.* 38, 977 (1955). Toxicity studies: F. C. Lu, A. Lavalle, *Can. pharm. J.* 97, 30 (1964); W. H. Hansen *et al., Food Cosmet. Toxicol.* 4, 389 (1966). *See also: Colour Index* vol. 4 (3rd ed., 1971) p 4385.



A dull, dark green powder, or a bright, crystalline solid. Sol in water to a green soln which becomes brownish-yellow on addn of HCl and blackish-green with NaOH. An excess of NaOH decolorizes the soln. Sparingly sol in alcohol; it dissolves in concd H_2SO_4 to a yellow soln which, when diluted with water, turns first yellowish-red, then green. LD_{50} orally in rats: > 2 g/kg (Lu, Lavalle).

USE: Limited use as a dye for silk and wool fabrics; as biological stain. Delisted by FDA in 1966 for use in foods, drugs, and cosmetics.

4604. D-Gulonic Acid. $C_6H_{12}O_7$; mol wt 196.16. C 36.74%, H 6.17%, O 57.09%. Prepd as the sodium salt by reduction of sodium glucuronate with sodium amalgam in alkaline medium: Fischer, Piloty, *Ber.* 24, 525 (1891); from D-gulonic acid γ-lactone: Rehorst, Naumann, *Ibid.* 77, 24 (1944).

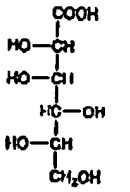


$[\alpha]_D^{20}$ −6° (10 min) → −38.6° (15 days). The free acid forms the lactone spontaneously. pK (25°): 3.68.

Sodium salt, $C_6H_{11}NaO_7$, crystals. $[\alpha]_D^{20}$ +11.5°. Sol in water.

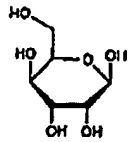
Calcium salt, $Ca(C_6H_{11}O_7)_2$. $[\alpha]_D^{25}$ −14.45° (c = 1.73). Precipitated from aq soln by ale.

4605. L-Gulonic Acid. Xylonecarboxylic acid. $C_6H_{12}O_7$; mol wt 196.16. C 36.74%, H 6.17%, O 57.09%. Prepd from L-xylose and HCN followed by hydrolysis of the nitrile: Fischer, Stahel, *Ber.* 24, 529 (1891). Prepn from D-glucuronic acid: Ger. pat. 618,907 (1935 to Hoffmann-La Roche); from L-gulonolactone: Ishidate *et al., Chem. Pharm. Bull.* 13, 173 (1965).



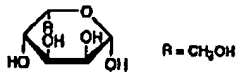
Crystallizes as the lactone on evapn of an aq soln.
Sodium salt, $[\alpha]_D^{20}$ +12.7° (c = 9). Freely sol in water.

4606. D-Gulose. $C_6H_{12}O_6$; mol wt 180.16. C 40.00%, H 6.71%, O 53.28%. Prepd by sodium amalgam reduction of an acid soln of the γ-lactone of D-gulonic acid: Fischer, Stahel, *Ber.* 24, 532 (1891); van Ekenstein, Blanksma, *Rec. Trav. Chim.* 27, 3 (1908). Alternate synthesis: Meyer zu Reckendorf, *Angew. Chem. Int. Ed.* 6, 177 (1967); *idem, Methods Carbohyd. Chem.* 6, 129 (1972); R. Köster *et al., Angew. Chem. Int. Ed.* 19, 547 (1980).



Syrup. Sweet taste. $[\alpha]_D^{20}$ −20.4°. Sol in water, slightly sol in alcohol. Not fermentable by yeast.

4607. L-Gulose. $C_6H_{12}O_6$; mol wt 180.16. C 40.00%, H 6.71%, O 53.28%. Prepd by sodium amalgam reduction of an acid soln of the γ-lactone of L-gulonic acid: Fischer, Piloty, *Ber.* 24, 526 (1891). *See also* van Ekenstein, Blanksma, *Rec. Trav. Chim.* 27, 3 (1908); Levene, LaForge, *J. Biol. Chem.* 20, 430 (1915); Talen, *Rec. Trav. Chim.* 44, 891 (1925); Isbell, *J. Am. Chem. Soc.* 55, 2167 (1933). Synthesis from D-mannose: Evans, Parrish, *Carbohyd. Res.* 28, 359 (1973); from D-glucose: D. K. Minster, S. M. Hecht, *J. Org. Chem.* 43, 3987 (1978).



Syrup. $[\alpha]_D^{20}$ +61.6°. $[\alpha]_D$ +21.3° (c = 4.58) (Evans, Parrish). Freely sol in water; slightly sol in alcohol. Not fermentable by yeast.

4608. Gum Benzoin. Resin benzoin; resin benjamin; gum benjamin. Balsamic resin from *Styrax benzoin* Dryand., known as Sumatra benzoin, or from *S. tonkinensis* (Pierre) Craib, *Styracaceae*, or other species of *Styrax* known as Siam benzoin. *Habit.* Thailand, Cambodia, S. Vietnam, Sumatra, Java, and Sunda Islands. *Constit.* Ethereal oil, free and combined benzoic and cinnamic acids up to 30%, vanillin, coniferyl benzoate, resin (a mixture of benzoresinol and benzoresinotannol) esterified with benzoic acid, styrol, styracin. Not less than 90% of Siam and not less than 75% of Sumatra benzoin is sol in ale (U.S.P.). *Ref:* Reinitzer, *Arch. Pharm.* 264, 131 (1926); Brans, *Pharm. Weekbl.* 73, 374 (1936); Freudenberg, Büttner, *Ber.* 83, 600 (1950).

USE: Preserving ointments; preparing natural benzoic acid; for fumigating pastilles; in perfumery and cosmetics.

THERAP CAT: Topical protectant.

THERAP CAT (VET): Tincture is used topically as an antiseptic and to promote healing; as an inhalant for bronchitis, and orally as an expectorant.

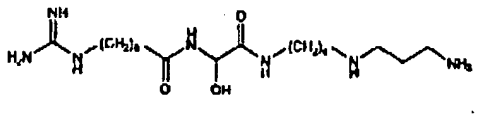
4609. Gum Tragacanth. Tragacanth. Mol wt about 840,000. The dried gummy exudation from *Astragalus gummifer* Labill. (white gavan) or other Asiatic species of *Astragalus, Leguminosae*, found largely in Iran, also in Asia Minor and in Syria. When mixed with water gives a soluble fraction, as a hydrosol, called *tragacanthin* which is a complex mixture of polysaccharides containing D-galacturonic acid, other sugars, and traces of starch and cellulose. The insoluble fraction swells to a gel and consists of 60-70% bassorin, *q.v.* Structural studies: Norman, *Biochem. J.* 25, 200 (1931); James, Smith, *J. Chem. Soc.* 1945, 739, 749; Aspinall, Baillie, *ibid.* 1963, 1702, 1714. *Reviews:* Bench, in *Natural Plant Hydrocolloids*, Advances in Chemistry Series 11 (A.C.S., Washington, 1954) pp 38-44; Meer *et al.* in *Industrial Gums*, R. L. Whistler, Ed. (Academic Press, New York, 2nd ed., 1973) pp 289-299. *Book:* F. Smith, R. Montgomery, *The Chemistry of Plant Gums and Mucilages* (Reinhold, New York, 1959) 627 pp.

Odorless. Insipid, mucilaginous taste. Acid reaction. One gram requires 0.9 ml 0.1*N* NaOH for neutralization to phenolphthalein: Gabel, *J. Am. Pharm. Assoc.* 23, 341 (1934). Viscosity of tragacanth mucilages is reduced by adding acid, alkali, and NaCl particularly if the mucilage is heated: Mantell, *The Water-Soluble Gums* (New York,

1947). Maximum initial viscosity of solns at pH 8; maximum stable viscosity near pH 5. Forms a deep yellow stringy precipitate when a soln is boiled with a few drops of 10% aqueous ferric chloride soln. A stringy precipitate formed also on heating a soln with Schweitzer reagent. Tragacanth is entirely insol in alcohol.

USE: In pharmaceutical compounding and dispensing, e.g., to suspend heavy insol powders, as an excipient for tablets and to impart consistence to troches; also in making emulsions and emulsifying agents; as stabilizer, thickener, texturizer in food; in adhesives (mucilages, pastes); in textile sizing, textile printing and general printing inks, and in dyeing with insol color lakes.

4610. Gusperimus. *(±)-7-[(Aminoiminomethyl)amino]-N-[2-[[4-[(3-aminopropyl)amino]butyl]amino]-1-hydroxy-2-oxoethyl]heptanamide;* (±)-*N*-[[[4-[(3-aminopropyl)amino]-butyl]carbamoyl]hydroxymethyl]-7-guanidinoheptanamide; 1-amino-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione; deoxyspergualin; (±)-15-deoxyspergualin. $C_{17}H_{37}N_9O_3$; mol wt 387.53, C 52.69%, H 9.62%, N 25.30%, O 12.39%. Synthetic derivative of the antitumor antibiotic *spergualin*. Prepn: Belg. pat. 894,651; H. Umezawa *et al.*, U.S. pat. 4,518,532 (1983, 1985 both to Microbiochem. Res. Found.); Y. Umeda *et al., J. Antibiot.* 38, 886 (1985). Prepn of the active (−)-isomer: H. Iwasawa *et al., ibid.* 35, 1665 (1982); H. Umezawa *et al.*, Eur. pat. Appl. 94,632; *eidem*, U.S. pat. 4,525,299 (1983, 1985 both to Microbiochem. Res. Found.). Synthesis and bioactivity of isomers: Y. Umeda *et al., J. Antibiot.* 40, 1316 (1987). Mechanism of action study: W. E. G. Müller *et al., Ibid.* 1028. HPLC determn in plasma: R. Nakanuma *et al., J. Chromatog.* 527, 208 (1990). Pharmacokinetics: J. P. Muindi *et al., Cancer Res.* 51, 3096 (1991). Preliminary evaluation in renal transplant rejection: H. Amemiya *et al., Transplant. Proc.* 25, 730 (1993). Series of articles on synthesis and immunomodulating activity: *Ann. N.Y. Acad. Sci.* 685, 123-206 (1993).



Trihydrochloride, $C_{17}H_{37}N_9O_3.3HCl$. *BMS-181173, NKT-01, NSC-356894, Spanidin.* White powder, prepd as the sesquihydrate. Sol in water. pH of 50 mg/ml saline soln: ~4.9. LD_{50} in mice (mg/kg): 25-50 i.p. (Umezawa).

(−)-Form trihydrochloride, prepd as the dihydrate. Colorless syrup, no def mp. $[\alpha]_D^{25}$ −7.3° (c = 1 in H_2O). LD_{50} in mice (mg/kg): 35-40 i.v. or i.p. (Iwasawa).

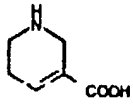
THERAP CAT: Immunosuppressant.

4611. Gutta-Percha. The purified, coagulated, milky exudate of various trees of the genus *Palaquium, Sapotaceae. Habit.* Malayan Archipelago. Extensive review: Williams, *Econ. Bot.* 18, 5-26 (1964). Defined as a *trans* isomer of rubber. Rubber has a repeat period of 8.2 Å, whereas α-gutta-percha has 8.7 Å and β-gutta-percha has 4.8 Å. The short period of β-gutta-percha identifies it almost uniquely as an all-*trans* polyisoprene.

Becomes pliable at 25-30°, plastic at 60°, mp 100° (partial dec). On exposure to air and sunlight, it absorbs oxygen and becomes brittle. Insol in water; partially sol in hot alcohol; 90% or more dissolves in chloroform, carbon disulfide, petr ether, oil of turpentine. *Keep under water and protected from light.*

USE: Insulator in electrotechnics, as dental cement; in orthopedics for fracture splints; manuf surgical instruments; covering golf balls.

4612. Guvacine. *1,2,5,6-Tetrahydro-3-pyridinecarboxylic acid; 1,2,5,6-tetrahydronicotinic acid.* $C_6H_9NO_2$; mol wt 127.14. C 56.68%, H 7.13%, N 11.02%, O 25.17%. From betel nuts, the seeds of *Areca catechu* L., *Palmae.* Extraction: Jahns, *Ber.* 24, 2615 (1891). Synthesis: Freudenberg, *ibid.* 51, 976, 1669 (1918); Hess, Leibbrandt, *ibid.* 51, 806; 52, 206 (1919).



Prisms from water, dec 295°. Neutral to litmus. Sol in water. Almost insol in abs alc, ether, chloroform, benzene.

Hydrochloride, $C_6H_{10}ClNO_2$, needles from water, dec 318°. Sol in water.

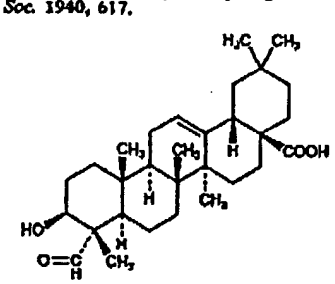
Hydrobromide, $C_6H_{10}BrNO_2$, needles from abs alcohol, dec 280°. Almost insol in acetone.

USE: Has been proposed as growth factor for *Staphylococcus aureus* and *Proteus vulgaris* instead of nicotinic acid.

4613. Gymnemic Acid. Gymnemin. Antisaccharin principle occurring as the potassium salt (gymnemin) in the leaves of *Gymnema sylvestre* R.Br. and allied *Asclepiodaceae*: Hooper, *Chem. News* 59, 159 (1889); Mhaskar, Caius, *Indian J. Med. Res.* No. 16, 1 (1930); Warren, Pfaffman, *J. Appl. Physiol.* 14, 40 (1959); Stöcklin *et al., Helv. Chim. Acta* 50, 474 (1967). A complex mixture of at least nine closely related acidic glycosides, the major active component being gymnemic acid A_1: Stöcklin, *J. Agr. Food Chem.* 17, 704 (1969); Dateo, Long, *ibid.* 21, 899 (1973). Separation of major components: Sinsheimer *et al., J. Pharm. Sci.* 59, 622, 629 (1970). Completely obtunds taste for several hours for bitter or sweet, e.g., quinine or sugar, but not for sour, astringent or pungent substances.

The acid is a yellow to brown amorphous, bitter powder. Almost insol in water; sol in alcohol. The potassium salt is a reddish-brown cryst mass, sol in water or alcohol.

4614. Gypsogenin. *(3β,4α)-3-Hydroxy-23-oxoolean-12-en-28-oic acid;* githagenin; albasapogenin; gypsophilasapogenin. $C_{30}H_{46}O_4$; mol wt 470.69. C 76.55%, H 9.85%, O 13.60%. From *Agrostemma githago* (L.) Scop., *Caryophyllaceae*: Wedekind, Kreeke, *Z. Physiol. Chem.* 155, 122 (1925); from *Gypsophila oldhamiana, Caryophyllaceae*: Kutani, Karr. *J. Pharm. Soc. Japan* 64, 18 (1944); *C.A.* 45, 2961d (1951). Structure: Ruzicka, Giacomello, *Helv. Chim. Acta* 20, 299 (1937). Identity with githagenin: Kon, Soper, *J. Chem. Soc.* 1940, 617.



Needles or leaflets from methanol, mp 274-276°. $[\alpha]_D$ +91° (alc).

Acetate, $C_{32}H_{48}O_5$, square tablets from methanol, mp 176-177°. $[\alpha]_D^{20}$ +78° ($CHCl_3$).

Methyl ester, $C_{31}H_{48}O_4$, crystals, mp 192°.

H

4615. H3e. Gerovital; Gero GH 3; Sex-Ex. A factor claimed to caine hydrochloride preps accor M.D. Anna Aslan [Institute of G is Dr. Aslan's name for the facto she found to be effective in achievi phenomena previously considered encountered in cerebral arteriosc *Weekly*, Dec. 6, 1959, p 36, Aslan Historical review of controversy *Youth Doctors* (Coward-McCann, 1 192; A. Hecht, *FDA Consumer* (Ma study: M. R. Hall *et al., Age Ageing* response: P. H. Millard, *Br. Med.*

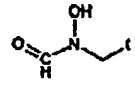
Note: Oral version is marketed. nule contains: Procaine hydrochl phyrin 0.0002 g, magnesium carbo

4616. Hachimycin. *Trichomy* nat. Heptaene macrolide antibiot *Streptomyces hachijoensis* from soil chijo Jima: S. Hosoya *et al., J. A* Yamaguchi, *ibid.* 7A, 10 (1954). insol derivs: S. Hosoya *et al.*, tha time, trichomycin was believed to and hamycin, q.v.: H. Burrows, 53, 566 (1970). Subsequent HPLC three polyene antibiotics to be diff *et al., ibid.* 189, 249 (1980). Ac *vaginalis, Treponema pallidum.* Tr yeast, weak activity against *Asperg* Yamaguchi, *J. Antibiot.* 7A, 10 (19 *et al., J. Antibiot.* 6A, 98 (1953). H. A. Lechevalier, *The Actinomyc* Wilkins, Baltimore, 1962) pp 397-

Yellow crystals. Acid reaction dium salt. Can be ppted as a wa derivs such as acriflavine, by sulfa nilamide, by basic antibiotics such enzymes such as papain and lys methylene blue, and by metallic s i.p. in mice: 0.05 mg/10-12 g mou

THERAP CAT: Antifungal. Antip

4617. Hadacidin. *N-Formyl-*N-hydroxyaminoacetic acid; N acid. $C_3H_5NO_4$; mol wt 119.08. 11.76%, O 53.74%. Antitumor an from cultures of *Penicillium freq* thesis: Knezka *et al., Biochemistr* Schoenewaldt, U.S. pat. 3,154,578 Biosynthesis: Stevens, Emery, *B. Review:* Shigeura, "Hadacidin" in P. Shaw, Eds. (Springer-Verlag, N 456.



Unstable crystals, mp 119-120°. fies on standing. The decompo and *N*-hydroxyglycine. Dibasic a ilon shows pH peak at 3.5 and 9.1 anol, ethanol, acetone, ether.

Monosodium salt, $C_3H_4NNaO_4$, hydrate, very freely sol in water.

4618. Hafnium. Hf; at. wt 1 4; also 2, 3. Six naturally occurrin 178 (27.1%); 177 (18.56%); 179 (1 (0.163%); α-emitter, $T_{1/2}$ 2 × 10^{15} 137; 158; 168-173; 175; 181-183 crust: 5 ppm. Found in all zircon covered in 1923 by Coster and H (1923). Extraction from the miner

Ca

Handbook of Water-Soluble Gums and Resins

ROBERT L. DAVIDSON
Editor in Chief

McGRAW-HILL BOOK COMPANY
New York St. Louis San Francisco Auckland Bogotá
Hamburg Johannesburg London Madrid Mexico
Montreal New Delhi Panama Paris São Paulo
Singapore Sydney Tokyo Toronto

Library of Congress Cataloging in Publication Data

Main entry under title:

Handbook of water-soluble gums and resins.

Includes index.
1. Gums and resins—Handbooks, manuals, etc.
I. Davidson, Robert L.
TP975.H26 668'.37'0202 78-24007
ISBN 0-07-015471-6

1234567890 KPKP 89876543210

The editors for this book were Harold B. Crawford and Joseph Williams, and the production supervisor was Thomas G. Kowalczyk. It was set in Gael by The Kingsport Press.

Printed and bound by The Kingsport Press.

Gum tragacanth is also used in the confectionery industry as a binder in cough drops and lozenges, gum drops, jujubes, and pastilles.

Gum tragacanth is used in the pharmaceutical and cosmetic field in gynecological jellies, ointments and salve bases, syrup, fish and mineral oil emulsions, tablets, pills, cosmetic creams and lotions, facial clays, toothpastes, and hair dressings.

Industrially, gum tragacanth finds applications for textile sizing, printing pastes, furniture and auto polishes, crayons, ceramics, insecticide emulsions, and cigars.

Chemical Nature

Gum tragacanth as found in nature exists as a slightly acidic salt, a complex mixture of polysaccharides containing calcium, magnesium, and potassium. After acid hydrolysis, the major sugars produced are D-galacturonic acid, D-galactose, L-fucose, D-xylose, L-arabinose, and L-rhamnose.

Structure Most investigators believe that gum tragacanth consists of at least two components:[15,20] a water-swellable major component, bassorin (60 to 70%), and a water-soluble component, a colloidal hydrosol, tragacanthin.

There has been some confusion on the nomenclature of these two constituents, and one author[6,7] refers to the soluble part as an araban and the swellable part as tragacanthin, which is in direct conflict with the majority opinion.

The water-swellable bassorin contains the tragacanthic acid polymer, while the water-soluble portion, tragacanthin, is a neutral polysaccharide which, at best, contains only small amounts (3%) of uronic acid.[3] This finding is in contrast to an earlier work which shows uronic acid as a major constituent in the water-soluble portion.[13]

Also, this neutral polymer has been referred to in early works as a galactaraban,[22] but more recent work[3] refers to it as an arabinogalactan, and rightfully so, since the arabinose is the sugar found in abundance and galactose is the core repeating unit.

Therefore, hereafter in this chapter, the water-soluble, neutral polymer will be referred to as an arabinogalactan, and the water-swellable polymer as tragacanthic acid.

The neutral polysaccharide, arabinogalactan, may be separated from the tragacanthic polymer in an aqueous solution with the addition of ethanol. The arabinogalactan is soluble in ethanol at concentrations as high as 70%, while the tragacanthic acid will precipitate. It is not certain whether the arabinogalactan is in a physical admixture with, or chemically bonded to, the polysaccharide acid, although its ease of separation favors the former view.

The partial structure of tragacanthic acid is shown in Fig. 11.1.[3] The arabinogalactan is highly branched (although strict evidence of homogeneity is not available), and this suggests that the structure is based on a core of D-galactose residues to which are attached highly ramified chains of L-arabinofuranose residues.[3] The majority interior chains of D-galactopyranose are joined by 1,6 linkages, and the smaller portions by 1,3 linkages. The L-arabinofuranose residues are mutually joined by 1,2, 1,3, and 1,5 linkages.

Also, the general rule of thumb that the more highly branched a polysaccharide is, the more water-soluble it becomes, supports this reasoning, since the arabinogalactan is very water-soluble.

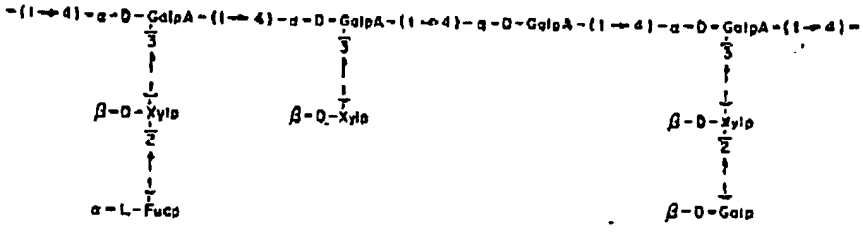


Figure 11.1 Structure of tragacanthic acid. *(Ref. 2.)*

(19) Japan Patent Office (JP)
(12) Laid-Open Patent Report

(11) Laid-open patent application no.
S57-7420
(43) Date laid open: January 14, 1982

(51) IPC: A 61 K 35/74
Identification code:
Internal control no.: 7138-4C

Inspection requested: No
Number of inventions: 1
(Total 5 pages)

(54) Antitumor agent

(21) Application no.: S55-81471
(22) Application date: June 18, 1980
(72) Inventor: Masashi Sasaki
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(72) Inventor: Kenjiro Kodama
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Specification

1. Title of Invention
Antitumor agent

2. Scope of Patent Claims
An antitumor agent that contains as an active ingredient a cell wall component of mold belonging to the *Aspergillus* genus.

3. Detailed Explanation of the Invention
This invention pertains to an antitumor agent that contains as an active ingredient a cell wall component of mold belonging to the *Aspergillus* genus.
In the past, several antitumor agents having fungus of microorganisms as an active ingredient have been reported, but all of them pertain to yeasts, bacilli or basidiomycetes, and the antitumor activity of fungus components of mold has not been reported.
As a result of various studies of the antitumor activity of the fungus components of mold, these inventors discovered that the cell wall component of mold belonging to the *Aspergillus* genus has antitumor activity, and they achieved this invention. That is, this invention offers an antitumor agent that contains as an active ingredient a cell wall component of mold belonging to the *Aspergillus* genus.
The "mold belonging to the *Aspergillus* genus" used in this invention is a microorganism belonging to asymmetric ascomycetes or the *Aspergillus* genus, whose cell wall components exhibit antitumor activity. Specific examples of molds belonging to the *Aspergillus* genus include *Aspergillus oryzae, Aspergillus sojae, Aspergillus tamarii, Aspergillus niger, Aspergillus usamii, Aspergillus saitoi, Aspergillus awamori, Aspergillus japonicus* and so forth. Among these, any strain that is compatible with the purpose of this invention can be selected.
The "cell wall component" that is the active ingredient of this invention is that prepared from the fungus of mold belonging to the *Aspergillus* genus. The cell wall is that which contains as a main ingredient a polysaccharide comprising at least glucose, mannose, galactose, N-acetyl glucosamine and so forth. As other sugar ingredients, the presence of ribose, arabinose, xylose,

laminaribese, glucosamine, galactosamine and so forth is known. For example, sample analysis values of the main composition of cell wall component prepared from *Aspergillus oryzae* F-1124 (Microorganism Laboratory fungus no. 1365) are shown below.

(Preparation method)

By nylon paste cultivation using soy bean–wheat as a culture medium, *Aspergillus oryzae* F-1124 was cultivated at 30°C for 72 hours, after which fungi were separated and removed from the top of the nylon cloth using a blade. The fungi were rinsed with deionized water, after which they were freeze-dried, and dried fungi were obtained. The dried fungi were pulverized by a dynomill, and they were stirred and extracted at 20°C for 3 hours using lauryl sodium sulfate. Then, they were stirred and extracted at 5°C for 16 hours, and the cell walls were centrifugally separated. After being rinsed, they were dialyzed and freeze-dried, and cell walls were thereby prepared.

(Analysis methods)

1) After the cell walls were hydrolyzed with sulfuric acid, neutral sugar was quantified by the Petri method, and this was expressed by glucose conversion.

2) After the cell walls were hydrolyzed with sulfuric acid, amino sugar was quantified by an amino acid automatic analyzer.

3) Crude protein was quantified by the Lowry-Folin method.

(Analysis values)

Neutral sugar quantity		61%
	Glucose	78.6%
	Mannose	13.9%
	Galactose	3.3%
	Ribose	5.4%
	Arabinose	3.8%
Amino sugar quantity		19.9%
	Glucosamine	88.0%
	Galactosamine	12.0%
Crude protein quantity		7.4%

The cultivation method of mold fungus to prepare the cell wall component, the separation method of the fungus, and the refinement method of the cell wall component are not particularly restricted. Known general methods can be suitably utilized, and processing methods for obtaining the suitable cell wall products can be devised appropriately depending on the purpose of this invention.

As the cultivation method of the mold fungus, any good cultivation methods can be used, such as ordinary liquid cultivation methods, and solid cultivation methods using agar, nylon paste, asbestos or sponge as the cultivation base, and mixed cultivation methods in which the two are combined. In the case of liquid cultivation methods, either the liquid surface cultivation method or in-liquid cultivation method can be used, and among in-liquid cultivation methods, the shaking cultivation method or deep cultivation method can be used. As the culture medium for liquid cultivation, natural cultivation bases such as yeast syrup and wheat germ syrup, and synthetic cultivation bases such as Zabeck liquid, Laurin liquid and Benneberg liquid can be given as examples, but any culture medium in which *Aspergillus* genus microorganisms can be used, and a culture medium that results in good fungus production can be selected. As the culture medium for solid cultivation, soybeans, wheat, barley, rice, bread, corn, fish powder, leaf protein, microorganism protein, gluten and so forth can be used individually or in combination. In addition, inorganic salts, vitamins, minerals and so forth can be added to these culture media, and any culture medium can be used as long as *Aspergillus* genus microorganisms can be grown under any culture medium conditions.

As cultivation conditions, optimal conditions can be selected in accordance with the culture medium composition, cultivation method and fungus strain, but as an example, in the case where *Aspergillus oryzae* F-1124 is cultivated by shaking cultivation in a glucose peptone culture medium, the fungi are produced with good yield under cultivation conditions of 28°C for 72 hours.

Fungus is separated and removed from the cultivation matter by o ary methods, and a suitable separation method is selected depending on the fungus strain. For example, in the case of liquid cultivation, after cultivation is finished, centrifugal separation, filtration, tilting or pressure rods can be used.

The fungus is pulverized by physical methods using a homogenizer, dynomill, French press and so forth. To make pulverization easy, pretreatment by chemical methods, enzyme methods, organism methods and so forth can be performed in advance to an extent that does not harm the active ingredient. As chemical treatments, there are acid treatment by hydrochloric acid, sulfuric acid, acetic acid or citric acid, alkali treatment by sodium hydroxide, potassium hydroxide or calcium hydroxide, treatment by salts such as sodium chloride, or treatment by organic solvents such as alcohol, chloroform or acetone. After the fungus is immersed and stirred into each pharmaceutical solution, it is treated by heating and drying. In enzyme treatment, enzymes that do not harm the active ingredient are used to make pulverization of fungus easy, by cell wall softening, cell internal substance elution promotion and so forth. In physiochemical treatment, parts of animals or microorganism cells are used. For example, in the case of animals, gastric juices and intestinal juices are used, and in the case of microorganisms, fungus can be treated by immersion, stirring, heating or drying using protoplasts.

To prepare the cell walls from the pulverized fungus, elution of contents in the fungus is required. As the eluate, water, salt solutions (sodium chloride aqueous solution, potassium chloride aqueous solution and so forth), acids (trichloracetic acid, perchloric acid and so forth), alkalis (sodium hydroxide, potassium hydroxide and so forth), organic solvents (ether, hexane, methanol, chloroform and so forth), surface active agents (lauryl sodium sulfate, polyoxyethylene lauryl ether, glycerin fatty acid ester, sucrose fatty acid ester, sorbitan fatty acid ester and so forth) and so forth can be used individually, or in combinations of two or more. As for the treatment method and conditions for elution, any treatment method within a range that does not harm the active ingredient can be used, and no particular conditions are required. The simplest method is elution by stirring. In the case of elution by stirring, stirring and extraction for 3-6 hours at room temperature is sufficient. A cell wall component is obtained by centrifugal separation or filtration of the obtained fungus suspension, and the eluate is completely removed by rinsing or dialysis, and cell walls are obtained.

The rinsed cell walls are normally dried. As the drying method, spray drying, air drying, freeze drying, vacuum drying and so forth can be used, as long as they do not harm the active ingredient. The yield of the cell wall component is 30-40% by dry conversion in the case of *Aspergillus oryzae* F-1124.

The cell wall component prepared in this way can be used as is as the active ingredient of this invention, but it is preferable to perform a sterilization process. As sterilization processes, it is possible to use boiling, evaporation, drying, irradiation, ultraviolet rays and so forth. Sterilization conditions can be ordinary conditions; for example, evaporation sterilization on something suspended in phosphoric acid buffer solution can be performed at 119°C for 10 minutes.

The antitumor agent of this invention can be administered orally, by injection, directly into the tumor, or by other suitable administration methods. The method of producing the agent suitable for the administration method is an ordinary method. That is, depending on the purpose, whether oral or injection, any suitable agent type can be decided upon. In the case where the cell walls are in powder form, a dispersed agent, a tablet, or a liquid agent in which the powder is dispersed in a liquid dispersant can be produced.

The dosage of the cell wall component should be determined by a physician while taking into consideration the source origin of the cell walls, the preparation method, administration method, disease condition and condition of the patient, but it is generally 1-50 mg/kg of body weight per day.

Next, the antitumor effect and acute toxicity of the cell wall component are described. Furthermore, the cell wall component used in these tests was obtained by the following preparation method.

100 ml of a glucose peptone culture medium comprised of 5% glucose, 0.5% polypeptone, 0.05% monopotassium phosphate, 0.05% dipotassium phosphate, 0.04% magnesium sulfate and 0.04% calcium chloride of pH 5.6 was put into a 500-ml flask, and after *Aspergillus* genus microorganisms were planted, they were cultivated for 72 hours at 28°C while being shaken. After cultivation was finished, the fungus was separated using a glass filter, it was rinsed with sufficient water and freeze-dried, and fungus was obtained. The fungus was pulverized for 60 minutes at 0°C in a dynomill, and then stirred and extracted at room temperature for 10 hours with 100 times its amount of 1% lauryl sodium sulfate. After extraction by stirring, it was centrifugally separated at 12,000 rpm for 30 minutes at 15°C. Then it was rinsed with deionized water, and dialysis with flowing water was performed at 5°C for 48 hours. It was freeze-dried, and a cell wall sample was prepared.

(1) Antitumor effect

Test example 1

Cell walls prepared from *Aspergillus oryzae* F-1124, *Aspergillus tamarii* IFO4287 and *Aspergillus sojae* IFO4274 were suspended in phosphate buffer solution containing 0.5% carboxymethylcellulose so as to result in 5.0 mg/ml and 2.5 mg/ml, and these were sterilized by heating for 10 minutes at 119°C, thereby creating agents.

2×10^7 cells of sarcoma 180 were transplanted under the skin on the rear of ICR-JCL mice (male, 7 weeks old), and 25 mg or 50 mg per kg of body weight of the agent was intraperitoneally administered a total of 9 times, on the 1st, 3rd, 5th, 7th, 9th, 11th, 13th, 15th and 18th day after transplantation. On the 32nd day after administration was finished, tumor weight was measured. Mice in which absolutely no cancer tissue was seen were calculated as 0 g; mice in which traces were seen but were below the measurement limit of 0.1 g were all calculated as 0.1 g. These results are shown in Table 1.

Table 1

Tested agent	Dosage (mg/kg)	Tumor weight (g)	Average (g)	T/C (%)
Control	-	12.4, 12.0, 9.6, 9.2, 9.2, 9.1, 9.0, 8.4, 7.8, 7.2, 4.2, 4.1	8.5	100
Aspergillus oryzae F-1124 cell walls	25	0.5, 0.4, 0.2, 0.1, 0.1, 0.1, 0, 0, 0, 0, 0	0.18	1.5
	50	6.5, 1.9, 1.3, 0.4, 0.2, 0.1, 0, 0, 0, 0	1.04	12.2
Aspergillus tamarii IFO4287	25	9.5, 6.7, 0.4, 0.3, 0.1, 0.1, 0, 0, 0	1.90	22.4
	50	1.4, 0.1, 0.1, 0.1, 0.1, 0.1, 0	0.39	4.6
Aspergillus sojae IFO4274	25	6.0, 0.6, 0.1, 0, 0, 0, 0, 0, 0, 0	0.61	7.2
	50	2.2, 1.9, 1.1, 0.6, 0.4, 0.1, 0.1, 0, 0, 0	0.64	7.5

As a result of significance testing, there was a significant difference from the control group with $P < 0.005$ for all treated groups, and it was seen that there is a marked tumor growth prevention effect.

Test example 2

An antitumor effect test by oral administration was conducted as follows.

Cell walls of *Aspergillus oryzae* F-1124 were suspended in phosphate buffer solution in a proportion of 12.5 mg/ml, thereby creating an agent.

10^6 cells of sarcoma 180 were transplanted under the skin on the rear of ICR-JCL mice (female, 10 weeks old), and forced oral administration of 0.2 ml per 10 g of body weight of the mouse (25 mg/kg body weight) of the agent was performed 10 times, every other day starting 24 hours after transplantation. On the 4th day after the last administration, tumor weight was measured. These results are shown in Table 2.

Table 2

Tested agent	Tumor weight (g)	Average (g)	T/C (%)
Control	30.3, 24.7, 19.0, 24.5, 10.4, 16.0, 14.4, 8.6, 1.2	16.6	100
Aspergillus oryzae F-1124 cell walls	20.5, 18.0, 15.2, 8.0, 6.7, 4.2	12.2	73

Test example 3

Cell walls of *Aspergillus tamarii* IFO4287 and *Aspergillus sojae* IFO4274 were suspended in sterilized physiological saline solution in a homogenizer so as to result in 4.0 mg/ml, this was diluted by half, then it was boiled, thereby producing an agent.

10^6 cells of sarcoma 180 were transplanted under the skin on the right thigh of ICR-JCL mice (female, 7 weeks old), and 0.05 ml per 10 g of mouse body weight was injected under the skin on the rear for a total of 10 doses, once every other day starting the day after transplantation. On the 87th day after transplantation, the mice were anesthetized, cancer under the skin was cut out, and its weight was measured. These results are shown in Table 3.

Table 3

Tested agent	Tumor weight (g)	Average (g)	T/C (%)
Control	12.3, 8.0, 8.0, 17.2, 11.3, 15.0, 14.7	12.4	100
Aspergillus tamarii IFO4287	5.8, 5.7, 15.1, 14.2, 14.5, 14.7, 0	10.0	81
Aspergillus sojae IFO4274	0, 0, 0, 0, 0, 15.0	2.5	20

(2) Acute toxicity

Acute toxicity in mice was as follows.

Female ICR-JCL mice, 10 weeks old, body weight 30-32 g were used. The administration methods used were oral and intraperitoneal. The presence of death and general symptoms were observed for 7 days after administration of *Aspergillus oryzae* F-1124 cell walls. As a result, no cases of death were seen even at the maximum dosage that could be technically administered, and LD_{50} was more than 500 mg/kg body weight in oral administration, and more than 200 mg/kg body weight in intraperitoneal administration.

Patent Applicant: (677) Yamasa Shoyu Co., Ltd.

Formal Correction Form (voluntary)
March 30, 1981

To: Patent Office Director-General

1. Display of article
   Patent application no. S55-81471

2. Title of invention
   Antitumor agent

3. Party making correction
   Relationship to article: Patent applicant
   Address: 10-1 Niifucho 2-chome, Choshi City 288
   Name: (677) Yamasa Shoyu Co., Ltd.
   Representative: Y. Hamaguchi

4. Object of correction
   Detailed explanation of the invention section of Specification

5. Contents of correction
   1) On the last line of page 2 of the Specification, insert "indicates a component of cell walls that has antitumor effect, that" between "The "cell wall component" that is the active ingredient of this invention is one that" and "is prepared from the fungus of mold belonging to the *Aspergillus* genus."
   2) On the second line of page 9 of the Specification, correct "The cell wall component prepared in this way can be used as is as the active ingredient of this invention, but it is preferable to perform a sterilization process." to "The cell wall component prepared in this way can be used as is as the active ingredient of this invention, but it can also be used after refining the active ingredient further from the cell walls. When administering this cell wall component, it is preferable to perform a sterilization process."
   3) On page 10 of the Specification, insert the following text between the 19th and 20th lines (before (1) Antitumor effect).
   "Also, in test example 4, the cell walls were extracted for 3 hours by 1N sodium hydroxide, and this was repeated 3 times, then centrifugal separation was performed at 5°C for 20 minutes at 12,000 rpm, and the supernatant was neutralized to pH 7.0 with acetic acid. Centrifugal separation was performed again, and the residue was re-suspended in deionized water, and dialysis with flowing water was performed for 48 hours at 5°C, then it was freeze-dried, and an active ingredient sample was produced."
   4) On page 14 of the Specification, insert the following text after the last line.
   "Test example 4

   An active ingredient sample of *Aspergillus oryzae* F-1124 was suspended in phosphate buffer solution containing 0.5% carboxymethylcellulose so as to result in 2.5 mg/ml, and this was sterilized by heating at 119°C for 10 minutes, thereby producing an agent.

   2×10^7 cells of sarcoma 180 were transplanted under the skin on the rear of ICR-JCL mice (male, 7 weeks old), and 25 mg per kg of body weight of the agent was intraperitoneally administered a total of 10 times on the 1st day after transplantation. On the 30th day after administration, tumor weight was

measured. T/C (%) was 2.5%, and the total tumor disappearance ratio was 80%."

(19) Japan Patent Office (JP)
(12) Laid-Open Patent Report

(11) Laid-open patent application no.
S59-112922
(43) Date laid open: June 29, 1984

(51) IPC:	Identification code:	Internal control no.:
A 61 K 31/715	ADP	7169-4C
// A 61 K 35/74	ADP	7138-4C
35/78	ADP	7138-4C
C 08 B 11/12		7133-4C
37/00		7133-4C

Inspection requested: No
Number of claims: 1
(Total 3 pages)

(54) Blood sugar reduction agent

(21) Application no.: S58-186304
(22) Application date: March 9, 1981
(62) Application no.: Part of S56-33513
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(74) Agent: M. Ariga, Patent Attorney, and 2 other parties

Specification

1. Title of Invention
Blood sugar reduction agent

2. Scope of Patent Claims

1. A blood sugar reduction agent characterized by containing as an active ingredient a saccharide selected from a group comprised of polysaccharides that are difficult to digest produced by plants or animals, and derivatives thereof.
2. A blood sugar reduction agent stated in item 1 of the Scope of Patent Claims in which the blood sugar reduction agent is a prevention or treatment agent of a metabolic disease.
3. A blood sugar reduction agent stated in item 1 of the Scope of Patent Claims in which the blood sugar reduction agent is a prevention or treatment agent of a digestive system disease.

3. Detailed Explanation of the Invention
This invention pertains to a new blood sugar reduction agent.
In recent years, metabolic diseases such as high blood sugar, diabetes, obesity and arteriosclerosis due to excessive eating and drinking of saccharides that are easy to digest and absorb, particularly starches and sugars, have been increasing even in Japan. Also, eating habits that tend toward such saccharides that are easy to digest and absorb have become a cause of digestive system diseases such as diarrhea, gastrointestinal catarrh and abnormal enzyme production in the intestines.
However, satisfactory prevention and treatment agents for such diseases have not been offered, and the invention of such has been desired.

Thus, as a result of earnest research, [redacted]s inventor discovered that polysaccha[redacted]s and oligosaccharides that are difficult to digest produced by plants or animals, and derivatives thereof, have an excellent blood sugar reduction effect, and thereby achieved this invention.

That is, this invention offers a blood sugar reduction agent that contains as an active ingredient a saccharide selected from a group comprised of polysaccharides that are difficult to digest produced by plants or animals, and derivatives thereof.

Examples of polysaccharides that are difficult to digest produced by plants or animals, and derivatives thereof, include cellulose, carboxyl methyl cellulose, methyl cellulose, ethyl cellulose, nitro cellulose, hydroxy ethyl starch, carboxy methyl starch, mannan, pectin, pectic acid, chitin, chitosan, carboxy methyl chitosan, glycol chitosan, aloe viscous matter, chondroitin sulfate, hyaluronic acid, heparin, luminalin, alginic acid, propylene glycol ester alginate, agar, gum arabic, arabinogalactane, carrageenan, dammar gum, karaya gum, kauri gum, locust bean gun, mastic gum, pontianac [?] gum, storax gum, traganth gum, plantain seed gum, inulin, chitin, xylin, galactomannan, tamarind seed viscous matter, quince seed viscous matter, flax seed viscous matter, okra viscous matter, gingko nut viscous matter and so forth. These saccharides and their derivatives are already publicly known and can be prepared by publicly known methods ("General Polysaccharide Science," last volume (K. Harada, A. Misaki, editors, pp. 172-436, 1974, Kodansha).

The saccharides of this invention can be crude products rather than refined products. The toxicity of the relevant saccharides is a very low 500 mg/kg or less by oral administration, and they can be independent or in a suitable composition, and they can be capsules, pills, liquids or injectable agents. Also, a safety agent can be added to these saccharides, and agents that prevent or treat metabolic diseases or digestive system diseases can also be added. In addition, the relevant saccharides can be used together with preservatives or food additives or foods such as concentrated foods, enzyme-producing foods, animal feeds, fish feeds, health foods and so forth.

It has already been found by this inventor that enzymes that synthesize saccharides that are difficult to digest from saccharides that are easy to digest have a blood sugar reduction effect (A. Endoh, Japan patent application no. S55-41390), and the saccharides of this invention can be used together with these enzymes or amylase or sucrase inhibitors.

The blood sugar reduction agent of this invention can be administered orally, intraperitoneally or intravenously, but oral administration is generally preferable. The dosage depends on the type and degree of the disease, but normally oral administration of 0.1-10 g/day, or in particular 0.2-5 g/day, is preferable.

Implementation examples of this invention are explained below.

Implementation example 1

Male Wister rats of body weight 145-180 g were starved for 24 hours, then given oral administration of 2 g/kg of sugar. At the same time, sugar that was dissolved or suspended in physiological saline solution was administered, and blood was taken from the tail vein after 30 minutes and after 1 hour, and the blood sugar value (blood glucose value) was measured by ordinary methods. As a result, as shown in Table 1, the group that received administration of a difficult-to-digest polysaccharide or oligosaccharide or derivative thereof had a lower blood sugar value than the control group that received administration of only sugar (the numerical values in the table are the average of 5 rats in each group).

Table 1

Sugar	Dosage (mg/kg)	Blood sugar reduction ratio (%) (a)	
		After 30 minutes	After 60 minutes
Konjak mannan	5	<10	<10
Citrus pectin	5	42	18
Carboxy methyl cellulose	5	<10	<10
Guar gum	5	<10	<10
Carrageenan (made from *kappa*)	5	<10	<10
Glycol chitosan	5	67	46

(a) Rise in blood sugar value 30 minutes and 60 minutes after sugar administration (2 g/kg) is used as a reference.

Implementation example 2

The blood sugar reduction effect of various saccharides was studied by the same method as in implementation example 1 (however, instead of sugar, 1 g/kg of starch that was heated and dissolved was orally administered). As a result, as shown in Table 2, a marked effect was seen for many saccharides.

Table 2

Sugar	Dosage (mg/kg)	Blood sugar reduction ratio (%) (a)	
		After 30 minutes	After 60 minutes
Konjak mannan	5	42	35
Citrus pectin	5	<10	<10
Carrageenan (made from *kappa*)	5	41	61

(a) Rise in blood sugar value 30 minutes and 60 minutes after starch administration (1 g/kg) is used as a reference.

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